REPORT

Y. Umezawa · T. Miyajima · H. Kayanne · I. Koike

Significance of groundwater nitrogen discharge into coral reefs at Ishigaki Island, southwest of Japan

Abstract Groundwater discharge from adjacent terrestrial areas can be a potentially important nutrient source to coastal coral reefs, since adjacent lands are often overlaid with permeable bedrock such as limestone. The quantity of groundwater nitrogen discharged into the Shiraho and Kabira coral reefs from their namesake watersheds on Ishigaki Island southwest of the Ryukyu Islands, Japan (24°19′–37′N, 124°4′–21′E) was monitored. These watersheds were subject to different types of nitrogen loading. The groundwater nitrogen discharge was compared by two independent methods, one based on measuring dissolved inorganic nitrogen (DIN) concentrations in the groundwater near the coastline, the other by estimating nitrogen loading from various land uses within the watershed. For a common watershed, the two methods agreed within a factor of two. The Shiraho reef received 4- or 5.5-fold more nitrogen than the Kabira reef. Groundwater discharge contributes significantly to the reef nitrogen budget, and is potentially a key factor controlling the biomass and succession of aquatic vegetation of the reefs.

Y. Umezawa (✉) · H. Kayanne
Department of Earth and Planetary Science,
Graduate School of Science,
University of Tokyo, Hongo 7-3-1,
Bunkyo-ku, Tokyo 113–0033, Japan

T. Miyajima · I. Koike
Marine Biogeochemistry Laboratory,
Ocean Research Institute,
University of Tokyo, Minamidai 1-15-1,
Nakano, Tokyo 164-8639, Japan

Present address: Y. Umezawa
Marine Biogeochemistry Laboratory,
Ocean Research Institute,
University of Tokyo, Minamidai 1–15–1,
Nakano, Tokyo 164–8639, Japan
e-mail: umezawa@ori.u-tokyo.ac.jp
Tel.: +81-3-53516525
Fax: +81-3-53516461

Introduction

The apparent discrepancy between the high productivity of coral reefs and the oligotrophy of the surrounding seawater has attracted the attention of researchers for decades (Odum and Odum 1955; Lewis 1977). However, many studies have shown the well-developed capacity of coral reef communities to take up dissolved and particulate nutrients from surrounding, oligotrophic seawater, as well as a capacity for nitrogen fixation (Webb et al. 1975; Wiebe et al. 1975; Wiebe 1985; Larkum et al. 1988; Larned and Atkinson 1997). In several reef systems, eutrophication resulting from anthropogenic nutrient inputs has been shown to alter community structure and species diversity of corals (Smith et al. 1981; Tomascik and Sander 1987a, 1987b; Grigg and Dollar 1991; Szmant and Forrester 1996; Hughes and Connell 1999; Wilkinson 1999).

Since coral reefs are frequently surrounded by highly porous, limestone drainage areas, the discharge of groundwater into coral reefs and its potential significance in eutrophication have been recognized by many researchers (Marsh 1977; Johannes 1980; D'Elia et al. 1981; Lewis 1985; Corbett et al. 1999). In uplifted tectonic areas like southwest Japan, many islands consist of Pleistocene limestone. These islands are surrounded by fringing reefs that developed during periods of stable sea level in the Holocene, and occasionally formed semi-closed shallow lagoons as a result of the development of a backreef area (Kan et al. 1997). Terrestrial discharge as groundwater is a potentially important nutrient source for such enclosed backreef areas (Crossland 1982).

To evaluate groundwater discharge into lakes and coastal waters, Lee (1977) used a seepage cylinder, a technique which, with various modifications, has since

been used in many settings for the estimation of nutrient discharge (Simmons 1992; Corbett et al. 1999; Rutkowski et al. 1999). The seepage cylinder is useful for nearshore areas whose hydrography results in significant submarine flux of groundwater from the sediments (Lee 1977). However, this method is difficult to apply to coral reefs where groundwater discharge occurs primarily above sea level at low tide, as is the case at Ishigaki Island, in the Ryukyu Islands. Here, groundwater input along the shorelines is evidenced, at low tide, by numerous intertidal rills and with well-developed brackish water flows. This is similar to the situation reported at Tumon Bay in Guam by Marsh (1977). Moreover, on Ishigaki Island it is difficult to measure groundwater flow rate and shore soil permeability by installing observation wells on the beach, since the shoreline consists of sandy beach with coral rubble. Therefore, to evaluate the nitrogen inputs through groundwater to coral reefs quantitatively, other reliable methods must be applied.

Since groundwater often contains high concentrations of dissolved nutrients (especially nitrate) derived from upland anthropogenic sources, the effects of groundwater discharge on the abundance and species composition of the benthic community in a backreef lagoon are potentially large. The waters around Ishigaki Island are characterized by the variety and extent of seaweed and seagrass coverage of its fringing reefs, and by the complex community structure of its nearshore areas (Ohba and Aruga 1982; Tanaka 1999). Although the distribution of seagrass may be controlled by many environmental and biological factors (Fonseca and Kenworthy 1987; Rutkowski et al. 1999), the offshore reef environments of the island are rather similar in that

they are strongly influenced by the Kuroshio Current. Rainfall, nitrogen fixation, and offshore water contributions to the nutrient supply appear to differ little among these reefs. By contrast, land uses on watersheds behind the backreef area differ markedly, partially reflecting the topography of the island.

Although many studies have reported nutrient inputs to waters of embayments including coral reefs (Alexander and Stevens 1976; Lewis 1985; Furnas et al. 1995; Valiela et al. 1997), Buddemeier (1996) pointed out that the estimates of terrestrial nitrogen loading, especially through groundwater, had large uncertainties. The precise evaluations of the amount of groundwater and its associated materials discharged into the coastal zone remain important topics in this field (Burnett and Turner 2001). In this study, we evaluated the groundwater nitrogen inputs to two different coral reefs using two different methods of estimating nitrogen loading, one based on dissolved inorganic nitrogen (DIN) concentrations in groundwater, the other on land use of the adjacent watershed. We hypothesized that the estimated values must be considered to be real if the amounts of terrestrial nitrogen input calculated by two different approaches agreed well, despite several uncertainties inherent in each method.

Materials and methods

Study site

Ishigaki Island is situated in the southwest portion of the Ryukyu Islands, Japan (24°19′–37′N, 124°4′–21′E), and is surrounded by fringing reefs 0.5–2.0 km wide. These include the two study sites, Shiraho and Kabira (Fig. 1). In contrast to the flat topography of

Fig. 1 Map of Ishigaki Island showing the location of **a** Shiraho and **b** Kabira watersheds (*shaded*), with the wells (*points*), and sampling area of nearshore water (*arrows*). Contour lines are drawn at 100-m intervals. Coral reefs are shown by the *fine line*; Shiraho and Kabira Reefs are *hatched*
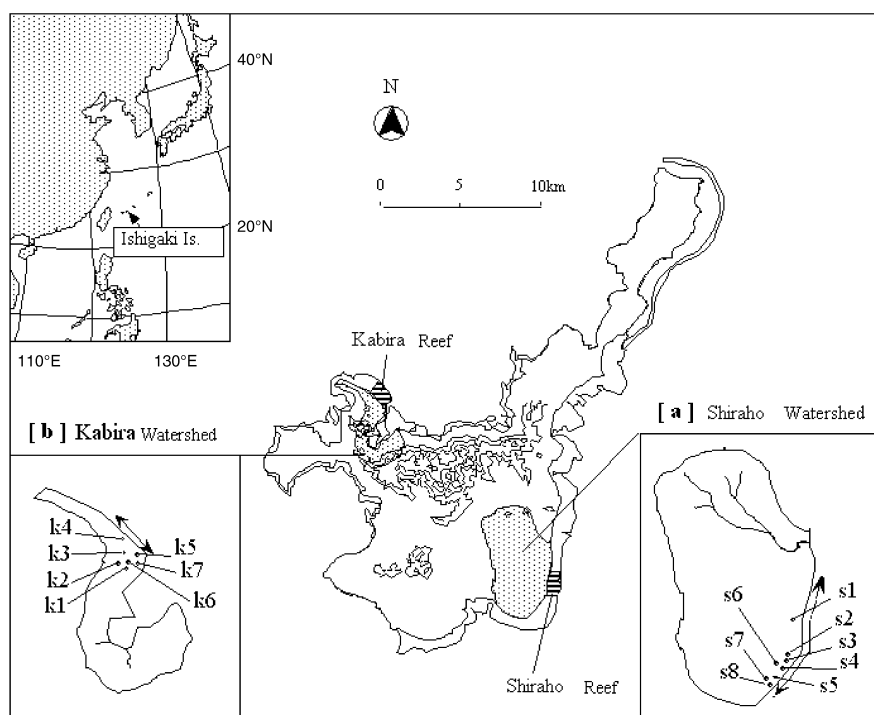
the southern area, low mountains (maximum 526 m above MSL) range across the central region of the island from east to west, from which steep and gentle slopes extend north and south, respectively. The climate is subtropical, with the annual mean temperature, relative humidity and precipitation being 24.3 °C, 78%, and about 2,000 mm, respectively. Two rainy seasons occur, monsoon (March to May) and typhoon (July to September). The dominant wind directions are southerly in summer and northerly in winter. Two reef sites were selected for which nitrogen loading as well as the size and hydrographic structure of the upland watershed were markedly different. There are a few fresh or brackish water wetlands at both areas.

Shiraho watershed and reef

The Shiraho watershed is situated to the southeast of Ishigaki Island (Fig. 1), and its area (21.96 km^2) was measured by digitizing a 1/25,000 geomorphologic map on a map digitizer (HTG-3648S; Hitachi Seiko Ltd.). The geology is mainly limestone and gravel belonging to the Pleistocene Ryukyu group (Foster 1965). This area has a high water permeability, and there is only one major river (Todoroki River) at the northern end of the watershed. Groundwater is consequently one of the two major forms of fresh water in this watershed, particularly as one travels south away from the mouth of the Todoroki River (Kawahata et al. 2000). The elevation of this area ranges from 0 to 80 m above MSL, and is gently undulating. Land use is mainly pasture with cow barns, sugarcane (*Saccharum officinarum* L.) or pineapple (*Ananas comosus* Merr.) fields, and some villages. Shiraho Reef, offshore from a portion of this watershed, is one section of the continuous fringing reef. The Shiraho Reef is about 0.70 km from shoreline to reef crest in the north and 0.85 km out from the shore in the south. It stretches 1.64 km along the shore. The southern boundary of the Shiraho Reef is exposed at low tide, and blocks the flow of seawater between the Shiraho Reef and the adjacent reef track (Nakamori et al. 1992). Near the shoreline where the water depth at mean sea level is about 1.0 m, dense seagrass beds (mainly *Thalassia hemprichii* and *Cymodocea* spp.) occur year-round. Their substrate is characterized by a mean sediment grain size of 0.5-1.0 φ, comprised of 17.3% very coarse sand ($<-0.5\ \varphi$), 56.9% coarse sand (-0.5 to $1.5\ \varphi$), 19.0% fine sand ($1.5-4.0\ \varphi$), and 6.8% silt and clay ($>4.0\ \varphi$) [Tanaka 1999: $\varphi = -\log_2$ diameter (mm)]. From winter to spring, dense populations of macroalgae (such as *Ulva* spp., *Enteromorpha* spp., and *Monostroma* spp.) occur along the shoreline. The inner reef flat mainly consists of coral rubble and carbonate sand. Coral skeletons (mainly *Heliopora coerulea* and *Porites cylindrica*) and coralline algae are the source of the rubble and sediment (Nakamori et al. 1992).

Kabira watershed and reef

The Kabira watershed (8.82 km^2) is situated in the northwest portion of Ishigaki Island (Fig. 1). The geology of the watershed is a mixture of non-permeable intruded granite and permeable limestone and gravel (Foster 1965). Fresh water reaches the coast through both groundwater and small rivers. The elevation of the catchment ranges from 0 to 300 m. Small mountains rise from the sea, with a narrow coastal plain supporting rice fields and a small village. A well-developed fringing reef is located along the coast. This portion of the reef stretches 1.8 km along the shore, and is 0.8 to 1.0 km from the shoreline to the reef crest. Kabira Reef also represents a section of the continuous fringing reef (Fig. 1). The southern boundary of Kabira Reef is the deep Kabira Channel (Yamano et al. 1998). In contrast to Shiraho Reef, the development of macroalgae and seagrass communities along the coastline is poor, but the water depth (about 1.0 m at mean sea level) and sediment grain sizes are similar. The mean sediment grain size is 0.5–1.0 φ, comprised of 24.8% very coarse sand ($<-0.5\ \varphi$), 53.7% coarse sand (-0.5 to $1.5\ \varphi$), 19.4% fine sand ($1.5-4.0\ \varphi$), and 2.2% silt and clay ($>4.0\ \varphi$) (Tanaka 1999). A diverse community of corals and algae extends from the inner reef to the crest (Yamano et al. 2000).

Estimating the terrestrial nutrient input

Two methods were applied to estimate terrestrial DIN inputs from the watershed to the reef through groundwater (Table 1). Method I estimated nitrogen loads by multiplying the dissolved inorganic nitrogen (DIN) concentrations in the groundwater along the coast by the groundwater mass flux into the lagoon. The groundwater flux was estimated by a water balance calculation using the meteorological data from the watershed. In method II, the nitrogen loads from urban industrial centers and agricultural activities within the watershed were summed, and the nitrogen discharge into the reef was calculated by correcting for possible loss (e.g. denitrification and ammonium volatilization) during the transport.

Table 1 Estimation of nitrogen loading of fringing reef systems from adjacent watersheds

Method I

$$N_{gi} = P \times A \times R \times [DIN] \qquad (1)$$

where

N_{gi}	Nitrogen inputs to reefs through groundwater (kg N/year)
P	Precipitation (mm/year)
A	Area of watershed (km^2)
[DIN]	DIN concentration in ground water (μM)
R	Groundwater discharge to precipitation ratio, ranging from 0.08–0.26[a]

Method II

$$N_{gi} = F + W \qquad (2)$$

where

F	Fertilizer applied to agricultural lands and pasture (kg N/year)[b]
W	Nitrogen reaching groundwater via wastewater from cesspools (kg N/year)

$$F = [FR - PNU] \times CA \times 0.5 \times 0.61 \times 0.39 \times 0.65^c \qquad (3)$$

where

FR	Crop fertilization rate (kg N ha^{-1} year^{-1})
PNU	Plant nitrogen uptake (kg N ha^{-1} year^{-1})[d]
CA	Cultivated area (ha)

$$W = AW \times POP \times (0.60\ to\ 0.90) \times 0.66 \times 0.65^e \qquad (4)$$

where

AW	Animal waste, N released per person or animal per year
POP	Human or animal population

[a]The variable ratio of groundwater discharge to precipitation is calculated as 0.5× (precipitation – evaporation) for each month (Table 2); 0.5 is the groundwater discharge ratio obtained from literature (see text)
[b]Atmospheric inputs over the surface area of the watersheds were trivial and consequently omitted
[c]The value 0.5 is the groundwater discharge ratio, 0.61 the fraction of N not lost as gases, 0.39 the fraction of N not lost in plumes, and 0.65 the fraction of N not lost in the aquifer (Valiela et al. 1997). Values of nitrogen removed as crops were ignored in this study, because most crops are consumed locally or recycled for organic fertilizer within the watersheds
[d]The value of 0.60–0.90 is the fraction of N not lost in cesspools. Since septic systems are not installed everywhere in this area, the fraction not lost in domestic cesspools was estimated at 0.60–0.90 (refer to Japanese Soil Association), which is rather large compared to the value of 40% used in Valiela et al. (1997). Wastewater in cesspools was considered to be directly filtered into the ground. The value of 0.66 is the fraction of N not lost in plumes and 0.65 the fraction not lost in the aquifer (Valiela et al. 1997)

Groundwater calculation: method I

Groundwater samples for DIN measurements were collected from wells along the shoreline (Fig. 1a,b) in March, June, September, and November 1997. Eight active wells, uncontaminated by surface waters (i.e. rainfall and wastewater), were selected in the Shiraho area and seven wells (including two springs) in the Kabira area. Water samples for DIN analysis were collected using a 2-L Van-Dorn water sampler (Rigousha Co. Ltd., Tokyo) and transferred to capped 10-mL acrylic tubes and stored in a freezer for later analysis. Analyses were performed within 3 weeks. The DIN concentrations were considered to represent those in the groundwater discharging into the shoreline, assuming there is no major nitrogen source or sink between these wells and the shoreline.

To estimate the annual groundwater discharge from the watershed, we used monthly weather data (precipitation and evaporation, January to December 1997) collected at the Ishigaki-jima Meteorological Observatory (Table 2). Annual nitrogen inputs to reefs through groundwater were calculated according to the method outlined in Table 1. The ratio of groundwater and river plus flash flood discharge from the watershed was assumed to be 1:1, as estimated from the mass flux of spring water on the adjacent Miyako Island, which is comprised mostly of limestone (Furukawa 1981).

Land-use calculation: method II

Groundwater DIN discharge to the reefs originated from a number of nitrogen sources in the watershed predominantly associated with human activities (i.e. fertilizer application and wastewater) (Table 1). Percentage nitrogen losses during transport were taken from Valiela et al. (1997), although nitrogen retention efficiencies of septic systems and sediments can vary between sites.

The populations of humans, livestock (cows), and fowl were obtained from the census data of 1997 (Table 3). Values of annual nitrogen release per head of population and livestock were taken from values obtained elsewhere in Japan (Table 3; Tabuchi and Takamura 1985).

The nitrogen fertilizer inputs per unit area due to various land uses, including the main crops of sugarcane and pineapple, were estimated from data supplied by the Farmers' Union (Table 4). For example, it takes 2–3 years to produce and harvest sugarcane, of which 0.5–1.5 years are a fallow period. About 3–4 years are required for pineapple. These crops receive several fertilizer applications. On Ishigaki Island, the rate of total fertilizer application (not including recycled-N from compost and manure) is 250 kg N/ha for sugarcane, twice the standard recommended rate (135 kg N/ha) for Louisiana, USA (Yaeyama Agricultural Agency, personal communication; Bengtson et al. 1998). For pasture, we used values for mainland Japan (Tabuchi and Takamura 1985). Manures (excreta and post-refining sugarcane refuse) are also applied to field crops. The Local Fertilizer Center produces about 4.3 Gg of organic fertilizer annually from 6.2 Gg of excreta and 7.1 Gg of sugarcane refuse (Local Fertilizer Center, unpublished data, July 1996–June 1997). We ignored these organic fertilizers and compost, because they were already accounted for in human- or animal-derived nitrogen, and the nitrogen removed by crops was also omitted from calculations (Table 1).

To measure watershed areas corresponding to different nitrogen source categories, aerial color photographs ($\approx$1: 11,500) taken in January 1995 were used to identify land uses, following the classification of Seher and Tueller (1973) and Madden et al. (1999). Extensive field observations were undertaken to verify the aerial photograph interpretations. Aerial photographs were digitized and land coverage was classified into nine land-use types: sugarcane fields, pineapple fields, other vegetables, rice fields, pasture, forest, uncultivated land, residential land, and open water. Photointerpretation keys were developed for each class (e.g. color, texture, tone, shape, etc.), through both field surveys and photo analysis. We also referred to the soil distribution map produced by the local Agricultural Cooperative Association, since some fields crops are predominantly grown on specific soil types.

Table 2 Monthly weather data (precipitation and evaporation) from Ishigaki-jima Meteorological Observatory (IMO) for Shiraho and Kabira watersheds, January–December 1997

	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
Precipitation (mm)													
Shiraho watershed	198	123	151	127	68	338	36	492	102	35	66	209	1,945
Kabira watershed	184	139	213	194	115	396	99	471	98	51	124	224	2,308
30-year average[a]	120	113	128	152	231	202	181	236	229	177	170	133	2,072
Evaporation ratio[b]	0.51	0.53	0.63	0.63	0.45	0.58	0.83	0.57	0.52	0.59	0.45	0.5	

[a] The 30-year average data are from Shiraho watershed
[b] The evaporation data were measured at the observatory

Table 3 Annual nitrogen release per individual, number of individuals, and total nitrogen loading calculated for Shiraho and Kabira watersheds

	Annual per capita excretion values for nitrogen (kg N individual^{-1} year^{-1})	Population densities		Estimates of annual total nitrogen inputs (kg N/year) from point sources to watershed surface	
		Shiraho	Kabira	Shiraho	Kabira
People	4.4	3,683	661	16,200	2,910
Cattle	106	2,106	440	223,200	46,640
Water buffalo	106	16	8	1,700	850
Horses	60	23	1	1,400	60
Swine	14.6	1,197	0	17,500	0
Goats	14.6	42	41	600	600
Poultry	0.73	10,000	0	7,300	0
Totals				267,900	51,060

Table 4 Annual nitrogen release per type of terrestrial area, size of each area, and total nitrogen loading calculated for Shiraho and Kabira. We accounted for a period in fallow, and did not included recycled organic fertilizer

	Estimates of annual fertilizer applications per hectare for each field (kg N ha^{-1} year^{-1})	Area for crops in both watersheds (and as percent of total area)				Estimates of annual total nitrogen inputs from point sources to watershed surface (kg N/year)	
		(ha)	(%)	(ha)	(%)		
		Shiraho		Kabira		Shiraho	Kabira
Sugarcane	125	765	(34.8)	60	(6.8)	95,590	7,530
Pineapple	338	106	(4.8)	10	(1.2)	35,610	3,480
Other vegetables	228	34	(1.6)	1	(0.1)	7,820	300
Rice field	103	34	(1.6)	10	(1.1)	3,520	1,000
Pasture	16	303	(13.8)	65	(7.4)	4,840	1,040
Forest	0	265	(12.0)	685	(77.6)	0	0
Uncultivated land	0	541	(24.6)	19	(2.1)	0	0
Residential area	0	117	(5.3)	22	(2.5)	0	0
Fresh-water bodies	0	32	(1.5)	11	(1.2)	0	0
Totals						147,380	13,350

Rainwater was sampled on several occasions from the Shiraho watershed, and analyzed for DIN concentrations to estimate the contribution of atmospheric deposition at the watershed level. We omitted those contributions, however, since rainwater DIN inputs were trivial compared to other nitrogen sources (i.e. wet deposition was 0.53–3.35 μM, about 0.1% of the total contribution of anthropogenic inputs).

Sampling of seawater along the shoreline

In September, just after the rainy period, samples of surface seawater were taken at 100-m intervals along the shorelines facing the watersheds (Fig. 1a, b). Each sampling point was about 0.5 m from the shoreline and 20–30 cm depth. Groundwater discharge from unconfined aquifers was often observed along the shorelines at low tide. Samples were handled and analysed as those obtained from inland wells.

Analytical methods

DIN concentrations ($NO_3^- + NO_2^- + NH_4^+$) were determined by Auto Analyzer II (BRAN + RUEBBE) according to the standard cadmium reduction and N–1-naphthylethylene diamine method for NO_3^- and NO_2^-, and the indophenol method for NH_4^+ (Scheiner 1976). Salinity was measured with a salinometer (SAT-2A, TOA Electronics Ltd.) having a precision of 0.1 psu.

Results

Groundwater flux: method I

A similar pattern of seasonal changes in total DIN concentration (NO_3^-, NO_2^-, and NH_4^+) was observed for both watersheds. Wells on the Shiraho watershed showed mean DIN concentrations above 400 μM in June (range 336–501 μM) and November (range 332–475 μM), and below 300 μM in March (range 267–309 μM) and September (range 155–291 μM) (Fig. 2, Table 5). Nitrate (vs nitrite and ammonium) accounted for 99% of the DIN. Similarity in DIN concentrations among the wells of the Shiraho basin [10.3% < CV (coefficient of variation) < 19.7%] suggests that its

drainage system is simple and uniform, whereas the lower values and wider variation (10.4% < CV < 77.1%) in DIN concentrations in the wells at Kabira suggest that this watershed is comprised of several independent drainage systems, each with its own nitrogen source.

In May, July, and September to November of 1997, precipitation was only 20–50% of the 30-year normal, while it was about double the norm in June, August, and December (Table 2). The DIN concentrations were inversely correlated with the integrated depths of precipitation during the preceding months (calculated from Tables 2 and 5)

As a first order of approximation, we assumed that precipitation during 3 months before sampling time was discharged through groundwater with the same DIN concentration as the sampled water (see 'Discussion'). Estimated groundwater nitrogen loading from the watershed was 35–50 Mg N/year (mean 43) at Shiraho and 3.5–18 Mg N/year (mean 11) at Kabira (Table 6). The
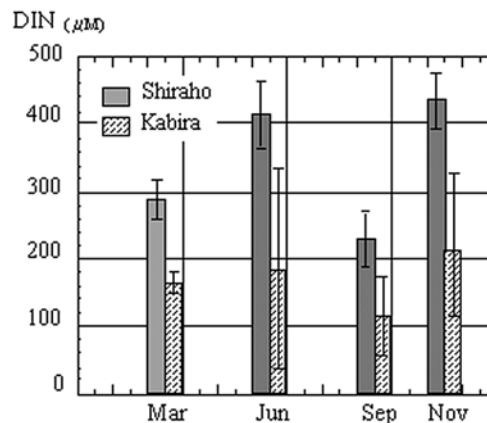
Fig. 2 Dissolved inorganic nitrogen (NO_3^-, NO_2^-, NH_4^+) concentrations in well waters adjacent to Shiraho and Kabira Reefs. Mean values and standard deviation (*bars*) of seven (at Kabira) or eight (at Shiraho) wells are plotted

Table 5 Dissolved inorganic nitrogen (NO_3^-, NO_2^-, NH_4^+) (µM) concentrations of wells adjacent to Shiraho and Kabira Reefs. Stations are shown in Fig. 1. *ND* No data

Shiraho

Station	March	June	Sept	Nov
s1	266.6	336	155.2	378.2
s2	308.5	423.9	291.2	433.1
s3	ND	413.5	228.1	475.4
s4	ND	500.7	274	412.8
s5	ND	391.9	216.5	434.5
s6	ND	430.9	194.3	449.2
s7	ND	ND	259.1	332.1
s8	ND	ND	205.5	ND
Average	287.5	416.1	228	416.5
±SD	±29.6	±53.7	±45.0	±47.8

Kabira

Station	March	June	Sept	Nov
k1	308.5	52.4	55.2	30.4
k2	ND	24.6	22.8	ND
k3	ND	301.2	203	278.1
k4	162.5	425.6	116.8	286.3
k5	188.5	179.4	182.1	232.2
k6	ND	192.3	84.1	151.9
k7	ND	ND	134.3	329.6
Average	175.5	195.9	114	218.1
±SD	±18.4	±151.0	±65.4	±110.0

Table 6 Estimates of nitrogen loading from Shiraho and Kabira watersheds. The range of estimation for method I is due to variability of nitrogen concentrations in the wells. *Numbers in parentheses* are mean values, obtained by substituting mean nitrate concentrations in groundwater into the computation of nitrogen loading. The margin of estimation for method II is attributable to the 10–40% variation in nitrogen loss from cesspools

	Shiraho area (Mg N/year)	Kabira area (Mg N/year)
Method I (groundwater method)	35–50 (43)	3.5–18 (11)
Method II (land-use method)	80–115	14–21

range of these values reflects the variation in DIN concentrations among the wells. This estimate indicates that groundwater DIN discharge at the Shiraho site is about four times greater than at the Kabira site.

Land-use flux: method II

The gentle slopes of the Shiraho watershed are used extensively for livestock farming of cattle and poultry, as well as for field crops of sugarcane and pineapple (Table 4). In comparison, 80% of the Kabira watershed is forested, a land use that releases very little nitrogen into the groundwater compared to other land uses (Howarth et al. 1996; Table 4). Furthermore, the population of the Kabira watershed is < 20% of that of the Shiraho watershed (Table 3).

The nitrogen load calculated to reach the reef through groundwater was 80–115 Mg N/year for the Shiraho watershed and 14–21 Mg N/year for the Kabira

watershed (Table 6). The margin of error for the estimated values reflects the variability in nitrogen losses in domestic cesspools. Method II estimates of groundwater nitrogen load were roughly twice those calculated by method I. However, the ratio of groundwater nitrogen loads in the Shiraho watershed to that in the Kabira watershed, estimated at 4.0 by method I, was similar to that estimated by method II (5.5).

Nitrate concentration and salinity along the shoreline

The nitrate concentration in seawater along the shoreline at low tide showed significant spatial variability, especially at Shiraho, possibly reflecting the uneven distribution of groundwater discharge points to the reef (Fig. 3). Indeed, fresh-water seepage was apparent at several locations along the sandy beach of Shiraho Reef. The nitrate concentrations observed at Shiraho Reef (range 3.3–230 µM; mean ± SD is 85.6±63.2) were generally much higher than those at Kabira Reef (range 1.5–55.3 µM; mean ± SD is 14.0±12.9).

Seawater salinity along the shoreline at Shiraho and Kabira Reefs ranged from 12–33 and from 25–34 psu, respectively (Fig. 4). Nitrate concentrations and salinity showed a significant negative correlation at Shiraho ($r = -0.80$, $n = 33$, $P < 0.0001$), while there was weak correlation at Kabira ($r = -0.53$, $n = 22$, $P < 0.05$). This suggests that the groundwater flux is larger and/or the mixing rate of discharged groundwater with seawater is slower in the Shiraho backreef lagoon. The poor correlation at Kabira may be attributable to the greater variability in DIN concentrations of the original groundwater (Table 5).

Discussion

Globally, terrestrial discharge of nitrogen to coastal waters has increased considerably from pre-industrial levels, reflecting the increase in human activities such as fertilizer utilization and leguminous crop cultivation (Galloway et al. 1995). Land-based nitrogen sources can be categorized into those of anthropogenic origin (McClelland et al. 1997; McClelland and Valiela 1998) and those resulting from natural nitrogen fixation by legume-symbionts and terrestrial blue–green algae (Marsh 1977; D'Elia et al. 1981). Nitrogen loading estimated by method II targets mainly anthropogenic sources, since it is the summation of various human activities (Tables 3 and 4). Although method I does not distinguish between the two sources of nitrogen, the estimates based on method I were nearly the same as those based on method II for both watersheds. Presumably, nitrogen in the groundwater at both sites is mainly of anthropogenic origin. Although anthropogenic nitrogen loading to coastal waters has frequently been reported in industrialized mid-latitude regions with

Fig. 3 Seawater nitrate concentrations (μM) at 100-m intervals along the shoreline of **a** Shiraho Reef and **b** Kabira Reef at low tide
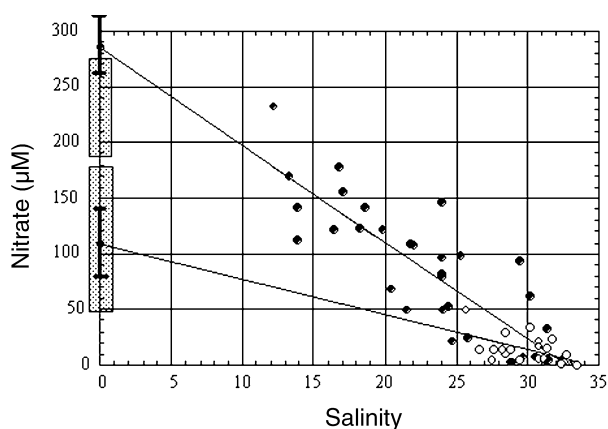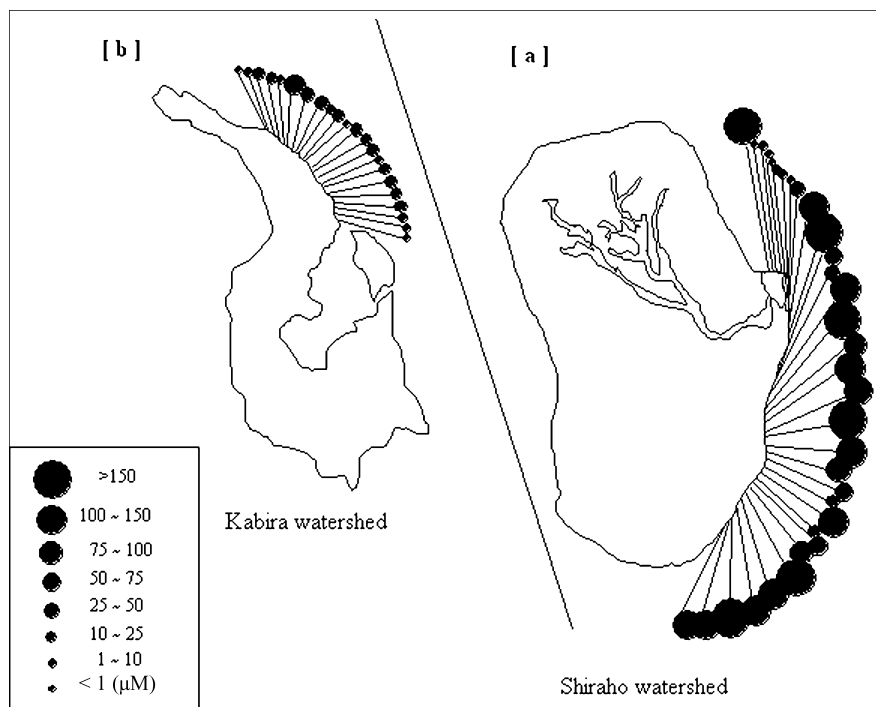

Fig. 4 Linear regression between nitrate concentration along the shoreline and salinity in nearshore water (*solid circles* Shiraho; *open circles* Kabira). Samples were collected at 100-m intervals along the shoreline. The salinity gradient indicates the degree of mixing. Intercept values extrapolated to salinity = 0 are 282.6±27.6 μM ($p < 0.0001$) for Shiraho and 104.0±32.4 μM ($p < 0.05$) for Kabira and putatively corresponded to nitrate concentrations in the original groundwater. *Areas within rectangles* on the ordinate indicate ranges of measured DIN concentrations in well waters of the Shiraho (mean 228±45 μM) and Kabira (mean 114±65 μM) watersheds in September (Table 5)

dense populations (e.g. Seitzinger and Kroeze 1998), coral reefs of tropical and subtropical regions with moderate to intensive land use of the inland watershed also suffer from terrestrial nitrogen loading (D'Elia et al. 1981).

Our results clearly indicate that terrestrial nitrogen inputs through direct groundwater discharge are significant in the vicinity of some coral reefs, and that the

volume of these discharges is controlled by the pattern of land use in the watershed. The methods applied in this study were first validated, and then used to compare the contribution of groundwater nitrogen discharge with that of other nitrogen sources in corresponding reefs. Possible effects of the groundwater DIN inputs on primary production and the types of vegetation are also considered.

Methodological considerations

The ratio of nitrogen discharge from the Shiraho watershed to that from the Kabira watershed yielded similar values when estimated by the two methods (4.0 vs 5.5), although absolute values of nitrogen discharge from method II were about double those calculated by method I (Table 6). The pros and cons of each method are outlined in Table 7, but it is difficult to assess their specific influence on estimates of nitrogen loading.

Nitrogen loads estimated by method II account for all nitrogen, including organic forms, whereas method I only accounts for DIN. In general, leaching losses of dissolved organic nitrogen (DON) vary between sites, coarsest soils having a more limited capacity to adsorb DON (Seely et al. 1998). Seely et al. (1998) and Correll et al. (1999) did show DIN and DON concentrations in fresh-water discharge to be of a similar order of magnitude. Because the DIN concentrations and temperatures measured in our study areas were much higher than those reported by them, DON in our study area may be converted to DIN, and thus form a lower proportion of total N. However, the gap in estimates

Table 7 Pros and cons of each method for estimating N loading through groundwater

	Benefits	Disadvantages
Method I	Logically simple	Uncertainty as to the ratio of groundwater discharge to precipitation: - Flood discharge during storm events cannot be estimated
	Approximated to real values, where DIN concentrations in the wells are considered to be close to that of real effluent groundwater to the reef	
	Applicable where census data are not available	- Differences between field-level evaporation and that measured at the observatory
		Uncertainty of real DIN concentrations of effluent groundwater: - Seasonal fluctuations of groundwater DIN concentrations - Heterogeneities for DIN concentrations among monitoring wells
		Uncertainty of boundary of watershed relating to the reefs
Method II	Convenient in relative comparison among sites	Possibilities of overestimation of N loading through groundwater: - Double-counting of crops and meat locally ingested in same watershed
	Field-approach unnecessary	- Overlooking of undegraded organic matter pooled in sediments
	Directly reflects the land uses on the watershed	- Extra flush-out of POM through surface water at storm event
	Applicable without groundwater sampling or calculation of water balance at the watershed level	Uncertainty of nitrogen loss estimations during transport

between method I and method II may be explained to some extent by the DON flux.

One major uncertainty associated with method I is attributable to seasonal changes in DIN concentrations in groundwater. Here, reservoir size and the residence time of groundwater in each watershed must be considered, which in turn influences the determination of an appropriate integrated period of precipitation to correspond to the DIN concentration. At Shiraho, the negative correlation between the DIN concentrations and the integrated precipitation during the preceding months became stronger, as a longer period (up to 60 days) was adopted for aggregating the precipitation ($r = -0.93$). At Kabira, a 60-day integrative period also gave a significant negative correlation ($r = -0.99$), although a significant correlation was also obtained with a 5-day integration ($r = -0.91$). Therefore, it would be valid to adopt the integrated precipitation during 3 months before sampling time in calculations with the same DIN concentration. Rainfall events were always reflected in groundwater DIN within a relatively shorter period at Kabira than at Shiraho, due to the smaller storage capacity at Kabira. Capone and Bautista (1985) working on the east coast of the United States showed better correlations between nitrate in groundwater and precipitation during the 30 days preceding sampling than that occurring 3 or 10 days before sampling.

Another uncertainty derives from the largely unpredictable variability in DIN concentrations in groundwater sampled from seepage or monitoring wells (Lee 1977; Uchiyama et al. 2000). Lewis (1985) argued that accurate estimates of DIN loading from groundwater discharge could not be made from DIN concentrations in the aquifer because of the large consumption of nitrate (e.g. denitrification and ammonium volatilization) between the monitoring wells and the groundwater

seepage. Our study, however, showed that the mean DIN concentrations in the monitoring wells near the coast were in good agreement with the extrapolated nitrate concentration at zero salinity calculated from the regression between nitrate and salinity from the nearshore water samples at both study sites (Fig. 4). Thus, the mean well water DIN concentrations measured at our study sites can be considered representative of concentrations in the groundwater seeping from the shoreline. Capone and Bautista (1985) also reported a similar correspondence for the interstitial water at Long Island and the upper aquifer in Suffolk County, New York, on the east coast of the United States.

Contribution of groundwater discharge to nitrogen in the lagoon and effects on marine flora

We estimated the other external sources of nitrogen input to the Shiraho and Kabira Reefs, and compared these to the land-derived nitrogen supplied through groundwater seepage (Table 8). Atmospheric depositions direct to the surface waters were estimated to be nearly the same (0.05–0.45 Mg N/year) for both Shiraho and Kabira, based on the DIN concentration in rainfall, the reef size and precipitation. Based on rates of nitrogen fixation reported for the other substratum of the reef, the total input from nitrogen fixation in the reefs was estimated at 0.8–1.7 Mg N/year for both reefs. On the other hand, the potential nitrogen inputs by advection from the open ocean and adjacent reefs were calculated to be 7.3–21.9 Mg N/year at Shiraho and 9.1–53.8 Mg N/year at Kabira. These values were obtained by multiplying the DIN concentration of offshore water by the inputs of offshore water (Umezawa 1998). The range of estimated values is a result of the variation in

Table 8 Estimations of nitrogen input from several sources to Shiraho and Kabira Reefs

External nitrogen sources	Shiraho Reef (1.34 km²)		Kabira Reef (1.42 km²)	
	Estimated nitrogen inputs (Mg N/year)	Contribution (%)	Estimated nitrogen inputs (Mg N/year)	Contribution (%)
Groundwater[a]	7.3–10.4	35.5	0.6–3.3	5.5
Offshore[b]	7.3–21.9	58.5	9.1–54.8	90.5
Nitrogen fixation[c]	0.88–1.67	5.1	0.80–1.51	3.3
Atmospheric depositions[d]	0.06–0.37	0.9	0.07–0.46	0.7
N needed in primary production[e]	20–45		20–45	

[a]Freshwater discharge was assumed to be proportional to length of shoreline. Estimations from method I were substituted

[b]Seawater inputs are $2.0–4.0\times10^6$ m³/day at Shiraho Reef and $0.25–1.0\times10^7$ m³/day at Kabira Reef (Yamano, personal communication, 2002), and DIN concentrations in seawater (offshore and adjacent reefs) were 10.0–15.0 µg N/L (unpublished data)

[c]Values ($\times10^{-4}$ kg N m^{-2} year^{-1}) of nitrogen fixation substituted in the calculation are 5.0–8.3 at bare sand, 8.7–16.0 at seagrass bed (Miyajima et al. 2001), 11.4–22.7 at inner reef community, 2.3–4.5 at sargassum and algal turf, 4.5–10.2 at outer reef flat (Larkum et al. 1988), and 7.3–16.4 by epiphytes on seagrass (Moriarty and O'Donohue 1993). Dimensions for each vegetation are from Iryu et al. (1991) and Yamano et al. (2000)

[d]Direct depositions on the water surface. DIN concentration of rainwater ranged from 0.53–3.35 µM (mean 1.61 µM, $n=4$) over four sampling dates. Inputs of DIN, DON, and dry deposition were all estimated to be of the same order of magnitude (Hinga et al. 1991; Morris 1991)

[e]Production data are from Iryu et al. (1991), Nakamori et al. (1992), and Kayanne et al. (1995), and C:N ratios are from Atkinson and Smith (1982)

the flux of offshore water due to wind direction and seawater DIN concentration. The volume of offshore water inputs at Shiraho is less than at Kabira, because of the longer periods of water ponding at low tide.

Groundwater discharge of nitrogen to the reef was estimated to be 7.3–10.4 Mg N/year at Shiraho and 0.6–3.3 Mg N/year at Kabira, assuming the discharge to be proportional to the length of shoreline. In this calculation, we used the values estimated by method I to obtain a conservative estimate. Estimated contributions of land-derived nitrogen through groundwater at Shiraho represented about 35% of total nitrogen inputs, but only about 5% at Kabira. These contributions may be overestimated if one considers groundwater discharges through confined aquifer occurring farther out, on the outer reef.

We also estimated the contribution of nitrogen from groundwater discharge to the nitrogen demands of primary producers on the reefs. The amounts of nitrogen needed in primary production were estimated to be 20–45 Mg N/year for both reefs (Table 8). If we simply assume a complete utilization of the discharged nitrogen by the reef vegetation, especially within the inner reef, the contribution of groundwater nitrogen discharge to the primary production in the reef ecosystem would be large (20–44%) at Shiraho, but small (4-10%) at Kabira. Due to the narrowness of the fringing reef (e.g. 700–1,000 m wide in our study area), the contribution of land-derived vs external nitrogen per reef area was relatively greater than that occurring in extensive, offshore reef systems, such as the Great Barrier Reef (Furnas et al. 1995). Johannes and Hearn (1985) reported that groundwater contributed half of the N requirements of a coastal lagoon off Perth, Western Australia. Of course, it is important to point out that N contributions to reef primary producers depend on not only the mass flux, but also the pathway of nitrogen inputs. For example, nitrogen introduced through nitrogen fixation is easily available to benthic organisms, whereas nitrogen delivered from the atmosphere to surface seawaters may be rapidly flushed out.

The evaluation of nitrogen loading to Shiraho Reef seems counterintuitive to the observation that nitrogen concentrations in the majority of lagoons were only slightly higher (0.4–1.4 µM N) than those in the offshore waters (0.2–0.8 µM N; unpublished data). Estimated turnover of the lagoon water was less than half a day (Nakamori et al. 1992; Yamano et al. 1998), suggesting rapid dilution of lagoon water with offshore waters. Also, the presence of dense seagrass beds near the shoreline may play a role in confining the effects of high DIN groundwater to nearshore regions of the lagoon through active nitrate uptake by the seagrasses and sedimentary denitrification (Miyajima et al. 2001). Therefore, DIN in groundwater discharge may not significantly affect the majority of coral reef biota and habitats, which are located some distance from the shoreline (Umezawa et al. 2002).

As for the relationship between the groundwater nutrient discharge and the biomass of aquatic vegetation, previous observations have been inconclusive. Some have found a positive relationship (Swell 1982; Lodge et al. 1989; Lapointe 1997; McCook 1999), while others have reported no correlation (Lillie and Barko 1990; Rutkowski et al. 1999). In our study sites, density of dominant seagrasses such as *Thalassia hemprichii* within 200 m of the coastline was 500–1,500 shoots/m² at Shiraho Reef, but only 0–100 shoots/m² at the Kabira Reef (Tanaka 1999). Since environmental factors such as texture of sediments and water depth at both seagrass

sites are similar (see 'Materials and methods'), the above difference in seagrass density may be caused by the difference in anthropogenic nutrient supplies. Crossland (1982) pointed to the high nitrogen concentrations in groundwater as a significant factor in the high biomass of macroalgae along the upper littoral fringe at Okinawa. In addition, seagrass bed sediments at Shiraho Reef were characterized by higher microalgal nitrate uptake than previously reported for seagrass beds in the tropics (Miyajima et al. 2001). Combining the above reports with our data on the difference in the groundwater DIN discharge between the two reefs suggests that land use and area of the adjacent watershed are responsible for the abundance and production of nearshore reef vegetation (e.g. seagrass and benthic algae). We conclude that land-derived nitrogen delivered through groundwater has an important role in the nitrogen cycle of the nearshore reefs.

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